

WHAT IS CLAIMED IS:

- 5 1. A compound of formula (I): R-linker-A-X, wherein R is one or more functional groups, wherein the linker is a multiatom straight or branched chain including C, N, S, or O, wherein A-X is a substrate for a dehalogenase, and wherein X is a halogen.

10 2. The compound of claim 1 which is a substrate for a *Rhodococcus* dehalogenase.

15 3. The compound of claim 1 wherein X is Cl or Br.

20 4. The compound of claim 1 wherein A is $(CH_2)_n$ and n = 4-10.

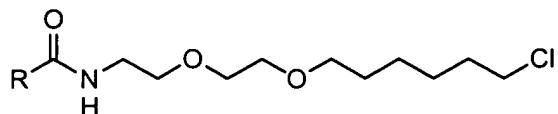
25 5. The compound of claim 1 wherein the linker comprises $(CH_2CH_2O)_y$ and y = 2-8.

30 6. The compound of claim 1 wherein the linker separates R and A by at least 12 atoms.

35 7. The compound of claim 1 wherein the linker comprises 3 to 30 atoms.

40 8. The compound of claim 1 wherein the linker has 11 to 30 atoms.

45 9. The compound of claim 1 which is



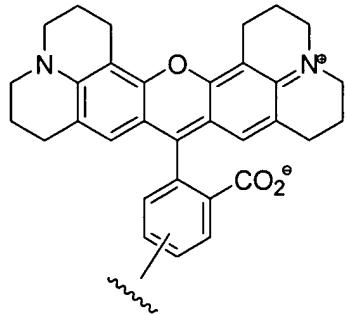
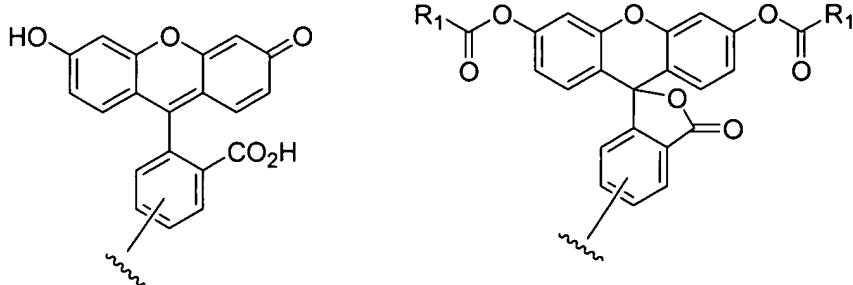
10. The compound of claim 1 wherein at least one functional group is an amino acid, protein, glycoprotein, nucleic acid molecule, drug, lipid, biotin, or solid support.

5 11. The compound of claim 1 wherein at least one functional group is an optically detectable molecule.

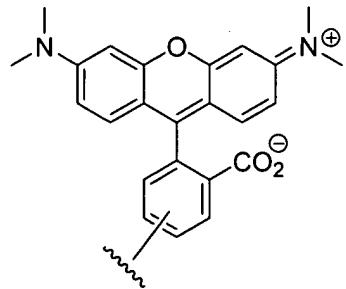
12. The compound of claim 11 wherein at least one functional group is a fluorophore.

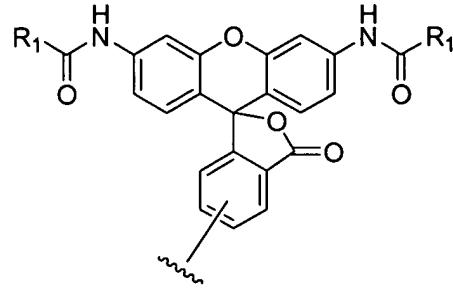
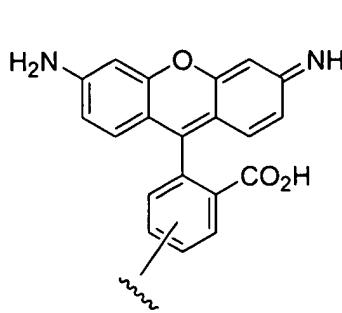
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13. The compound of claim 1 wherein R is one of



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and wherein R₁ is C₁-C₈.

- 14. The compound of claim 1 which comprises two functional groups.
- 5 15. The compound of claim 1 wherein at least one functional group binds Ca²⁺, binds K⁺, binds Na⁺, is pH sensitive, is a radionuclide, is electron opaque, is a chromophore, is a MRI contrast agent, fluoresces in the presence of NO or is sensitive to a reactive oxygen.
- 10 16. A mutant dehalogenase comprising at least one amino acid substitution relative to a corresponding wild-type dehalogenase, wherein the mutant dehalogenase forms a bond with a dehalogenase substrate which comprises one or more functional groups, which bond is more stable than the bond formed between the corresponding wild-type dehalogenase and the substrate, wherein the at least one amino acid substitution in the mutant dehalogenase is a substitution at an amino acid residue in the corresponding wild-type dehalogenase that is associated with activating a water molecule which cleaves the bond formed between the corresponding wild-type dehalogenase and the substrate or at an amino acid residue in the corresponding wild-type dehalogenase that forms an ester intermediate with the substrate, wherein the substituted amino acid at a residue associated with activating a water molecule is not glutamine or asparagine.
- 15 20 17. The mutant dehalogenase of claim 16 wherein the substitution is at a residue in the wild-type dehalogenase that activates the water molecule.
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18. The mutant dehalogenase of claim 17 wherein the residue in the wild-type dehalogenase that activates the water molecule is histidine.
- 5 19. The mutant dehalogenase of claim 16 wherein the substitution is at a residue in the wild-type dehalogenase which forms an ester intermediate with the substrate.
- 10 20. The mutant dehalogenase of claim 19 wherein the residue in the wild-type dehalogenase which forms the ester intermediate is aspartate.
- 15 21. The mutant dehalogenase of claim 16 wherein the at least one substitution is at a position corresponding to amino acid residue 272 of a *Rhodococcus rhodochrous* dehalogenase.
- 20 22. The mutant dehalogenase of claim 21 wherein the substituted amino acid at the position corresponding to amino acid residue 272 is phenylalanine, glycine or alanine.
- 25 23. The mutant dehalogenase of claim 16 wherein the at least one substitution is at a position corresponding to amino acid residue 106 of a *Rhodococcus rhodochrous* dehalogenase.
24. The mutant dehalogenase of claim 23 wherein the substituted amino acid at the position corresponding to amino acid residue 106 is cysteine or glutamate.
25. The mutant dehalogenase of claim 16 further comprising a protein of interest, thereby yielding a fusion protein.

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26. The mutant dehalogenase of claim 25 wherein the protein of interest is a selectable marker protein, membrane protein, cytosolic protein, nuclear protein, structural protein, an enzyme, an enzyme substrate, a receptor protein, a transporter protein, a transcription factor, a channel protein, a phospho-protein, a kinase, a signaling protein, a metabolic protein, a mitochondrial protein, a receptor associated protein, a nucleic acid binding protein, an extracellular matrix protein, a secreted protein, a receptor ligand, a serum protein, an immunogenic protein, a fluorescent protein, or a protein with reactive cysteines.
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27. The mutant dehalogenase of claim 16 which has at least 85% amino acid sequence identity to the corresponding wild-type dehalogenase.
28. A mutant serine beta-lactamase comprising at least two amino acid substitutions relative to a corresponding wild-type serine beta-lactamase, wherein the mutant serine beta-lactamase forms a bond with a serine beta-lactamase substrate which comprises one or more functional groups, which bond is more stable than the bond formed between the corresponding wild-type serine beta-lactamase and the substrate and the bond formed between a mutant beta-lactamase having one of the substitutions and the substrate, wherein the at least two amino acid substitutions in the mutant serine beta-lactamase are substitutions at amino acid residues in the corresponding wild-type serine beta-lactamase that are associated with activating a water molecule which cleaves the bond formed between the corresponding wild-type serine beta-lactamase and the substrate, and wherein the at least two amino acid substitutions in the mutant serine beta-lactamase are associated with the stability of the bond formed between the mutant serine beta-lactamase and the substrate.

29. The mutant beta-lactamase of claim 28 wherein the substitutions are at positions corresponding to amino acid residue 166 and amino acid residue 170 of a *Staphylococcus aureus* serine beta-lactamase.
- 5 30. The mutant beta-lactamase of claim 29 wherein the substituted amino acid at the position corresponding to amino acid residue 166 in the mutant beta-lactamase is an aspartic acid.
- 10 31. The mutant beta-lactamase of claim 28 wherein the substituted amino acid at the position corresponding to amino acid residue 170 in the mutant beta-lactamase is a glutamine.
- 15 32. The mutant beta-lactamase of claim 31 wherein the substituted amino acid at the position corresponding to amino acid residue 166 in the mutant beta-lactamase is an aspartic acid and the substituted amino acid at the position corresponding to amino acid residue 170 in the mutant beta-lactamase is a glutamine.
- 20 33. The mutant beta-lactamase of claim 28 further comprising a protein of interest, thereby yielding a fusion protein.
- 25 34. The mutant beta-lactamase of claim 33 wherein the protein of interest is a selectable marker protein, membrane protein, cytosolic protein, nuclear protein, structural protein, an enzyme, an enzyme substrate, a receptor protein, a transporter protein, a transcription factor, a channel protein, a phospho-protein, a kinase, a signaling protein, a metabolic protein, a mitochondrial protein, a receptor associated protein, a nucleic acid binding protein, an extracellular matrix protein, a secreted protein, a receptor ligand, a serum protein, an immunogenic protein, a fluorescent protein, or a protein with reactive cysteines.

35. A method to detect or determine the presence or amount of a mutant hydrolase, comprising:
- 5 a) contacting a mutant hydrolase with a hydrolase substrate which comprises one or more functional groups, wherein the mutant hydrolase comprises at least one amino acid substitution relative to a corresponding wild-type hydrolase, wherein the at least one amino acid substitution results in the mutant hydrolase forming a bond with the substrate which is more stable than the bond formed between the corresponding wild-type hydrolase and the substrate, wherein the at least one amino acid substitution in the mutant hydrolase is a substitution at an amino acid residue in the corresponding wild-type hydrolase that is associated with activating a water molecule which cleaves the bond formed between the corresponding wild-type hydrolase and the substrate or at an amino acid residue in the corresponding wild-type hydrolase that forms an ester intermediate with the substrate; and
- 10 b) detecting or determining the presence or amount of the functional group, thereby detecting or determining the presence or amount of the mutant hydrolase.
- 20 36. The method of claim 35 wherein the substitution is at a residue in the wild-type hydrolase that activates the water molecule.
37. The method of claim 36 wherein the residue in the wild-type hydrolase that activates the water molecule is histidine.
- 25 38. The method of claim 35 wherein the substitution is at a residue in the wild-type hydrolase that forms an ester intermediate with the substrate.
39. The method of claim 38 wherein the residue in the wild-type hydrolase that forms an ester intermediate with the substrate is aspartate.
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40. A method to isolate a molecule, cell or subcellular organelle of interest in a sample, comprising:
- 5 a) contacting a sample with a fusion protein comprising a mutant hydrolase and a hydrolase substrate which comprises one or more functional groups, wherein the mutant hydrolase comprises at least one amino acid substitution relative to a corresponding wild-type hydrolase, wherein the at least one amino acid substitution results in the mutant hydrolase forming a bond with the substrate which is more stable than the bond formed between the corresponding wild-type hydrolase and the substrate, wherein the at least one amino acid substitution in the mutant hydrolase is a substitution at an amino acid residue in the corresponding wild-type hydrolase that is associated with activating a water molecule which cleaves the bond formed between the corresponding wild-type hydrolase and the substrate or at an amino acid residue in the corresponding wild-type hydrolase that forms an ester intermediate with the substrate, wherein the fusion protein comprises a protein which binds a molecule, cell or subcellular organelle of interest; and
- 10 b) isolating the molecule, cell or subcellular organelle of interest.
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- 20 41. The method of claim 40 wherein the substitution is at a residue in the wild-type hydrolase that activates the water molecule.
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42. The method of claim 41 wherein the residue in the wild-type hydrolase that activates the water molecule is histidine.
43. The method of claim 40 wherein the substitution is at a residue in the wild-type hydrolase that forms an ester intermediate with the substrate.
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44. The method of claim 43 wherein the residue in the wild-type hydrolase that forms an ester intermediate with the substrate is aspartate.

45. The method of claim 40 wherein at least one functional group is a solid support or a molecule which binds to a solid support.
- 5 46. The method of claim 40 wherein the molecule of interest is a protein.
47. A method to label a cell, comprising:
a) contacting a cell comprising a mutant hydrolase with a hydrolase substrate which comprises one or more functional groups, wherein the mutant hydrolase comprises at least one amino acid substitution relative to a corresponding wild-type hydrolase, wherein the at least one amino acid substitution results in the mutant hydrolase forming a bond with the substrate which is more stable than the bond formed between the corresponding wild-type hydrolase and the substrate, wherein the at least one amino acid substitution in the mutant hydrolase is a substitution at an amino acid residue in the corresponding wild-type hydrolase that is associated with activating a water molecule which cleaves a bond formed between the corresponding wild-type hydrolase and the substrate or at an amino acid residue in the corresponding wild-type hydrolase that forms an ester intermediate with the substrate; and
b) detecting or determining the presence or amount of the functional group.
48. The method of claim 47 wherein the substitution is at a residue in the wild-type hydrolase that activates the water molecule.
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49. The method of claim 48 wherein the residue in the wild-type hydrolase that activates the water molecule is histidine.
50. The method of claim 47 wherein the substitution is at a residue in the wild-type hydrolase that forms an ester intermediate with the substrate.
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51. The method of claim 50 wherein the residue in the wild-type hydrolase that forms an ester intermediate with the substrate is aspartate.
- 5 52. The method of any one of claims 35, 40 or 47 wherein the wild-type hydrolase is a dehalogenase.
- 10 53. The method of claim 52 wherein the substrate comprises R-linker-A-X, wherein R is one or more functional groups, wherein the linker is a multiatom straight or branched chain including C, N, S, or O, wherein A-X is a substrate for a dehalogenase, and wherein X is a halogen.
- 15 54. The method of claim 53 wherein A is $(CH_2)_n$ and n = 4-10.
56. The method of claim 53 wherein the linker separates R and A by at least 12 atoms.
- 20 57. The method of any one of claims 35, 40 or 47 wherein the wild-type hydrolase is a serine beta-lactamase.
- 25 58. The method of any one of claims 35, 40 or 47 wherein the mutant hydrolase is present in a cell or on the surface of a cell.
59. The method of any one of claims 35, 40 or 47 wherein at least one functional group is an amino acid, protein, glycoprotein, nucleic acid molecule, drug, lipid, biotin, or solid support.
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60. The method of any one of claims 35, 40 or 47 wherein at least one functional group is an optically detectable molecule.
- 5 61. The method of claim 60 wherein at least one functional group is a fluorophore.
62. The method of any one of claims 35, 40 or 47 wherein the substrate comprises two functional groups.
- 10 63. The method of any one of claims 35, 40 or 47 wherein at least one functional group binds Ca^{2+} , binds K^+ , binds Na^+ , is pH sensitive, is electron opaque, is a chromophore, is a MRI contrast agent, is a radionuclide, fluoresces in the presence of NO or is sensitive to a reactive oxygen.
- 15 64. The method of any one of claims 35, 40 or 47 wherein the presence of at least one functional group in a cell is correlated to the subcellular location of the mutant hydrolase.
- 20 65. The method of any one of claims 35, 40 or 47 wherein the mutant hydrolase further comprises a protein of interest, thereby yielding a fusion protein.
- 25 66. The method of claim 65 wherein the protein of interest is a selectable marker protein, membrane protein, cytosolic protein, nuclear protein, structural protein, an enzyme, an enzyme substrate, a receptor protein, a transporter protein, a transcription factor, a channel protein, a phospho-protein, a kinase, a signaling protein, a metabolic protein, a mitochondrial protein, a receptor associated protein, a nucleic acid binding protein, an extracellular matrix protein, a secreted protein, a receptor ligand, a serum protein, an immunogenic protein, a fluorescent protein, or a protein with reactive cysteine.
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67. The method of claim 47 wherein the mutant hydrolase further comprises a selectable marker protein.
- 5 68. The method of claim 67 wherein at least one functional group in the substrate is a fluorophore.
69. The method of claim 68 wherein the mutant hydrolase forms an ester bond with the substrate.
- 10 70. The method of claim 68 wherein the mutant hydrolase forms a thioester bond with the substrate.
- 15 71. The method of claim 47 further comprising contacting the cell with a fixative prior to or after contacting the cell with the substrate.
72. The method of claim 47 further comprising contacting the cell with a fixative concurrently with contacting the cell with the substrate.
- 20 73. The method of claim 71 or 72 wherein the cell is fixed with methanol, acetone and/or paraformaldehyde.
74. The method of claim 67 further comprising contacting the cell with a fixative prior to or after contacting the cell with the substrate.
- 25 75. The method of claim 67 further comprising contacting the cell with a fixative concurrently contacting the cell with the substrate.
76. The method of claim 74 or 75 wherein the cell is fixed with methanol, acetone and/or paraformaldehyde.

77. The method of claim 52 wherein the mutant dehalogenase is encoded by a nucleic acid sequence which is optimized for expression in a selected host cell.
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78. A compound of any of formula II-XXVIII.
79. An isolated polynucleotide comprising a nucleic acid sequence encoding a dehalogenase, wherein the nucleic acid sequence for the dehalogenase is optimized for expression in a selected host cell.
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80. The isolated polynucleotide of claim 79 wherein the dehalogenase is a mutant dehalogenase comprising at least one amino acid substitution relative to a corresponding wild-type dehalogenase, wherein the mutant dehalogenase forms a bond with a dehalogenase substrate, which bond is more stable than the bond formed between the corresponding wild-type dehalogenase and the substrate, wherein the at least one amino acid substitution in the mutant dehalogenase is a substitution at an amino acid residue in the corresponding wild-type dehalogenase that is associated with activating a water molecule which cleaves the bond formed between the corresponding wild-type dehalogenase and the substrate or at an amino acid residue in the corresponding wild-type dehalogenase that forms an ester intermediate with the substrate.
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- 25 81. The isolated polynucleotide of claim 79 wherein the nucleic acid sequence hybridizes to SEQ ID NO:50 or the complement thereof under medium stringency conditions.

82. The isolated polynucleotide of claim 81 wherein the nucleic acid sequence hybridizes to SEQ ID NO:50 or the complement thereof under high stringency conditions.
- 5 83. The isolated polynucleotide of claim 79 wherein the nucleic acid sequence encodes a mutant dehalogenase with at least 85% amino acid sequence identity to the corresponding wild-type dehalogenase.
- 10 84. The isolated polynucleotide of claim 83 wherein the corresponding dehalogenase is encoded by SEQ ID NO:51.
85. The isolated polynucleotide of claim 79 wherein the nucleic acid sequence has less than 90% nucleic acid sequence identity to SEQ ID NO:51.
- 15 86. The isolated polynucleotide of claim 79 wherein the nucleic acid sequence has at least 90% nucleic acid sequence identity to SEQ ID NO:50.
- 20 87. An isolated cell comprising a polynucleotide encoding a fusion protein, wherein the fusion protein comprises a selectable marker protein and a protein which is capable of irreversibly or stably binding a substrate or a portion thereof which includes a functional group.
88. The cell of claim 87 wherein the functional group is a fluorophore.
- 25 89. The isolated cell of claim 87 wherein the protein which is capable of stably binding a substrate which includes a functional group is a mutant hydrolase comprising at least one amino acid substitution relative to a corresponding wild-type hydrolase, wherein the mutant hydrolase forms a bond with a hydrolase substrate which comprises a fluorophore, which bond is more stable than the bond formed between the corresponding wild-type hydrolase

and the substrate, wherein the at least one amino acid substitution in the mutant hydrolase is a substitution at an amino acid residue in the corresponding wild-type hydrolase that is associated with activating a water molecule which cleaves the bond formed between the corresponding wild-type hydrolase and the substrate or at an amino acid residue in the corresponding wild-type hydrolase that forms an ester intermediate with the substrate.

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90. The isolated cell of claim 87 wherein the protein irreversibly binds at least a portion of the substrate which includes the functional group.
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91. A method to label a cell, comprising:
contacting cells comprising a fusion protein comprising a selectable marker protein and a second protein which is capable of irreversibly or stably binding a substrate or a portion thereof which includes a functional group,
15 with the substrate.
92. The method of claim 91 wherein cells which express the selectable marker protein are selected prior to contacting the cells with the substrate.
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93. The method of claim 91 wherein cells which express the selectable marker protein are selected after contacting the cells with the substrate.
94. The method of claim 91 wherein the functional group is a fluorophore.
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95. The method of claim 91 further comprising contacting the cells with a fixative prior to or after contacting the cells with the substrate.
96. The method of claim 91 further comprising contacting the cells with a fixative concurrently with contacting the cells with the substrate.
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97. A mutant hydrolase comprising at least one amino acid substitution relative to a corresponding wild-type hydrolase, wherein the mutant hydrolase forms a bond with a hydrolase substrate which comprises one or more functional groups, which bond is more stable than the bond formed between the corresponding wild-type hydrolase and the substrate, wherein the at least one amino acid substitution in the mutant hydrolase is a substitution at an amino acid residue in the corresponding wild-type hydrolase that is associated with activating a water molecule which cleaves the bond formed between the corresponding wild-type hydrolase and the substrate or at an amino acid residue in the corresponding wild-type hydrolase that forms an ester intermediate with the substrate, wherein the substituted amino acid at a residue associated with activating a water molecule is not glutamine or asparagine.
98. The mutant hydrolase of claim 97 wherein the substitution is at a residue in the wild-type hydrolase that activates the water molecule.
99. The mutant hydrolase of claim 98 wherein the residue in the wild-type hydrolase that activates the water molecule is histidine.
100. The mutant hydrolase of claim 97 wherein the substitution is at a residue in the wild-type hydrolase which forms an ester intermediate with the substrate.
101. The mutant hydrolase of claim 100 wherein the residue in the wild-type hydrolase which forms the ester intermediate is aspartate.
102. The mutant hydrolase of claim 97 wherein the at least one substitution is at a position corresponding to amino acid residue 272 of a *Rhodococcus rhodochrous* dehalogenase.

103. The mutant hydrolase of claim 102 wherein the substituted amino acid at the position corresponding to amino acid residue 272 is phenylalanine or glycine.

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104. The mutant hydrolase of claim 97 wherein the at least one substitution is at a position corresponding to amino acid residue 106 of a *Rhodococcus rhodochrous* dehalogenase.

- 10 105. The mutant hydrolase of claim 104 wherein the substituted amino acid at the position corresponding to amino acid residue 106 is cysteine or glutamate.

106. The mutant hydrolase of claim 97 wherein the substituted amino acid at a residue associated with activating a water molecule is not methionine, aspartate, or alanine.

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107. A method for preparing a compound of formula R-Linker-A-X comprising coupling a compound of formula R-Y with a compound of formula Z-Linker-A-X, wherein Y and Z are groups that can react to link R- to – Linker-A-X.

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108. The method of claim 107 wherein R-Y is an activated ester of a compound of formula R and wherein Z is an amine suitable to react with the activated ester to form an amide bond.

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109. A method for preparing a compound of formula R-Linker-A-X wherein the Linker comprises an amide bond comprising coupling a corresponding activated ester with a corresponding amine to provide the compound of formula R-Linker-A-X.

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